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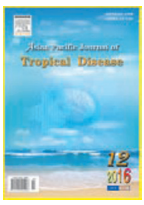

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Repellent activity of the creams formulated from *Annona senegalensis* and *Boswellia dalzielii* leaf fractions and essential oils against *Anopheles gambiae* (Diptera: Culicidae)

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ABSTRACT

Objective: To investigate the repellent efficacy of the creams formulated from methanol extract and *n*-hexane, chloroform, ethyl acetate and methanol fractions as well as essential oils of *Annona senegalensis* (*A. senegalensis*) and *Boswellia dalzielii* (*B. dalzielii*) leaves against the malarial vector *Anopheles gambiae* (*An. gambiae*) in the laboratory.

Methods: The efficacies of 25% w/w active ingredient creams formulated from the plant-based products were tested. Different concentrations of the creams, ranging from 2.0 to 12.0 mg/cm were applied on the exposed dorsal hand area (25 cm^2) of volunteers. The treated hands were submitted to 50 caged blood-starved females of *An. gambiae* for 3 min after every 30 min until 180 min.

Results: Total protection of up to 120 and 60 min without bites of *An. gambiae* were recorded with *n*-hexane creams applied at 12 mg/cm^2 respectively for *A. senegalensis* and *B. dalzielii*. The essential oil creams of the two tested plants applied at 6 mg/cm^2 protected volunteers up to 120 min without mosquito bites. The commercial Odomos cream (12% N,N-diethyl-3-methylbenzamide) tested as the positive control at 6 mg/cm^2 protected volunteers from mosquito bites up to180 min.

Conclusions: These results suggest that the cream formulated from the *n*-hexane fraction of *A. senegalensis* and essential oil creams of *A. senegalensis* and *B. dalzielii* leaves have the potential of a natural herbal source for the development of new, safe and eco-friendly repellent products to prevent *An. gambiae* bites.

1. Introduction

Mosquitoes belonging to the genera of *Anopheles*, *Aedes* and *Culex* have tormented humans with their bites and the transmission of some deadly diseases such as malaria, Dengue fever, Chikungunya, yellow fever and lymphatic filariasis[1]. In Africa, several *Anopheles* mosquito species are malaria vectors with the species *Anopheles gambiae* Giles (*An. gambiae*) being the most important and widely distributed one[2]. According to the World Health Organization[3], 584 000 people died in 2013 from malaria worldwide with 90% of these deaths occurring in Africa. In Cameroon, malaria is by far the leading cause of morbidity (15.6%) and mortality (13.0%)[4].To reduce the risk of malaria transmission in human populations, antimalarial drugs and vector control measures are emphasized[5]. Unfortunately, the disease still remains a threat for human health because of the unavailability of antimalarial vaccines and the inadequacy of vector monitoring in Africa. However, a suitable vector control measure for malaria prevention would be a part of the solution[6]. Among malaria control methods, the use of repellents is attractive, because it reduces contacts between mosquitoes and their hosts, and in turn, would lower the rate of disease transmission in many instances[7]. In this line, synthetic repellent products containing N,N-diethyl-3-methylbenzamide (DEET) which constitutes excellent repellent agents against mosquitoes and other biting insects are predominant in the market[8]. Unfortunately, these DEET based repellent scan cause irreversible damage to the ecosystem, as some of them are non-biodegradable. They may also cause skin irritations and unpleasant smell as well as discomfort owing to an oily feeling to some users[9,10]. The health and environmental problems associated with the use of synthetic repellents have forced researchers to seek for repellents based on other less hazardous chemicals. Therefore,

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The study protocol was performed according to the Helsinki declaration and approved by Anambra State University Teaching Hospital, Amaku, Awka, Anambra State, Nigeria Ethics Review Committee. Informed written consent was obtained from the students (2 women and 2 men without their identities) of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University Agulu, Awka, Nigeria.

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much interest is being focused on repellents of plant origin, because they are more biodegradable and less harmful to humans and domestic animals. Thus, several plant-based repellent formulations including inter alia *Cymbopogon citratus*, *Eucalyptus globulus*, *Curcuma longa*, *Azadirachta indica*, *Mentha piperita*, *Tribulus terrestris*, *Blumea lacera* were effective against *Anopheles*, *Aedes* and *Culex* mosquito species[11].

Annona senegalensis (*A. senegalensis*) (Annonaceae) is a bushy shrub or small tree, mostly found in Savannah and parts of the tropical rain forest regions of Africa[12]. As an insecticide, the leaf powder of *A. senegalensis* was toxic to *Sitophilus zeamais* Motsculsky (Coleoptera: Curculionidae)[13] and *Tribolium castenum* Herbst (Coleoptera: Tenebrionidae)[14]. Gueye *et al.*[15] reported the insecticidal activity of extract and essential oils of this plant against the groundnut weevil *Caryedon serratus* (Olivier) (Coleoptera: Bruchidae). Salifou *et al*.[16] reported the effectiveness of *A. senegalensis* against fleas and lice in poultry. On mosquito species, larvicidal activity of the fractions of this plant was demonstrated against *An. gambiae*[17] and *Aedes* aegypti (L.) (*Ae.* aegypti)[18].

Boswellia dalzielii (*B. dalzielii*) (Burseraceae) is a tree that grows up to 13 m in height, found in Savannah regions, and is locally abundant in central and west of Africa[19]. The larvicidal activity of the fractions of this plant was reported against immature stages of *An. gambiae* and *Culex quinquefasciatus* Say (*Cx. quinquefasciatus*) [17,20].

The objective of this study was to evaluate the repellent efficacy of the creams formulated from the leaf extracts/fractions and essential oils of *A. senegalensis* and *B. dalzielii* against adult female *An. gambiae*.

2. Materials and methods

2.1. Plant materials

The green leaves of *A. senegalensis* were collected from Dang (latitude 7°24'9.49" N, longitude 13°32'8.70" E and altitude 1 093 m above sea level), Ngaoundéré in the Adamaoua region of Cameroon on November 2011, while *B. dalzielii* leaves were collected from Midjivin (latitude 10°10'8.00" N, longitude 14°20'0.70" E and altitude 456 above sea level), Maroua, Far North region of the same country on December 2011. The plants were identified by Pr. Mapongmetsem Pierre Marie, a botanist of the Department of Biological Sciences in University of Ngaoundéré in Cameroon, and the identity were confirmed at the National Herbarium in Yaoundé, Cameroon, where voucher samples were deposited under the registration number 7783/SRF-CAM and 20532/SRF-CAM for *A. senegalensis* and *B. dalzielii*, respectively. The leaves were dried in a room under ambient conditions, then pulverized with an electric grinder and screened using 0.4 mm mesh size sieve. The powders were then stored at –18 ˚C in a deep freezer until they were needed for bioassay.

2.2. Extraction and fractionation

Extraction and fractionation of the leaf powders of *A. senegalensis* and *B. dalzielii* were carried out in the laboratory of the Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Nigeria during the period from March to May 2012. The initial extraction was processed with the methanol solvent to obtain the residue called methanolic crude extract (MCE).

To obtain the MCE, 1 200 g of the powders of each plant were macerated in 2 500 mL of methanol for 72 h at room temperature and then the maceration was filtrated using filter paper Whatman No.1. The residue of the maceration was rinsed and filtered several times with the fresh methanol until a clear phase was obtained. The filtrate was submitted to a rotary evaporator apparatus to obtain a residue called crude extract.

For the fractionation process, the method of Gueye *et al*.[15] was used. A total of 200 g of the crude extract of each plant was separated successively by the method of differential solubility in four solvents of different polarity: *n*-hexane, chloroform, ethyl acetate and methanol solvents. The crude extract was mixed with silica gel (70–260 mesh size) and macerated in *n*-hexane, then filtered with Whatman No.1 filter paper after phase separation. *n*-Hexane fraction and maceration (1) were recovered. Maceration (1) was dried in the open air and then soaked in chloroform; phase chloroform fraction filtrated and maceration (2) were also recovered. Maceration (2) after drying in the open air was soaked in ethyl acetate; phase ethyl acetate fraction filtered and maceration (3) were also recovered. Maceration (3) was finally taken up in methanol to recover the polar compounds in the methanol fraction after filtration. Each fraction was concentrated using a rotary evaporator and then stored at –4 ˚C in a refrigerator until needed for bioassay.

2.3. Extraction of essential oils

The finely-ground plant materials were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus. Distillates of essential oils were dried over anhydrous sodium sulfate, filtered and stored at –4 ˚C in a refrigerator until needed for bioassay.

2.4. Preparation of the repellent cream

The repellent cream for bioassay was formulated following the method used by Adeniran and Fabiyi[21]. Twenty-five percent (w/w) of the repellent cream product of each plant extract/fraction or essential oil were formulated. Pure white soft paraffin (8.0 g) was weighed in a 250 mL glass beaker and heated in a water bath at a temperature of about 50 ˚C. At this stage, 2.0 g of *A. senegalensis* or *B. dalzielii* extract/fraction or essential oil was added separately and mixed suitably. The mixture in a screwed covered bottle was stirred properly to ensure that the sample was uniformly mixed with the molten stage of the cream.

2.5. Collection and rearing of An. gambiae strain

The larvae of *An. gambiae* were collected from water sewage in gutters on February 2013 at Awka market in Anambra State of Nigeria and identified by the experts of the National Arbovirus Research Center, Enugu, Nigeria. To start the colony, the larvae were kept in plastic trays containing tap water. All the experiments were carried out at (27 ± 2) ˚C and 75%–85% relative humidity under 12:12 light-dark cycles. Larvae were fed a diet containing crayfish and biscuit in a ratio of 3:1, respectively. Pupae were transferred from the trays to a cup containing tap water and were maintained in insect cages (30 cm × 30 cm × 35 cm) where the adults emerged. The adults were maintained in cages and were continuously provided with 10% sucrose solution in a jar with a cotton wool. On day five, the adults were given a blood meal from a guinea pig shaved and placed on top of the cages overnight for blood feeding by females. Beaker with 100 mL of tap water lined with filter paper was kept inside the cage for oviposition. The sixth generation from the colony was used for the experiments.